# Dietary anaplerotic therapy improves peripheral tissue energy metabolism in patients with Huntington disease

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**Abstract**

We previously identified a systemic metabolic defect associated with early weight loss in patients with Huntington disease (HD) suggesting a lack of substrates for the Krebs cycle. Dietary anaplerotic therapy with triheptanoin is used in clinical trials to promote energy production in patients with peripheral and brain Krebs cycle deficit, as its metabolites – C5 ketone bodies – cross the blood brain barrier. We conducted a short-term clinical trial in six HD patients (UHDRS= 33 ±13, 15-49) to monitor the tolerability of triheptanoin. We also assessed peripheral markers of short-term efficacy that were shown to be altered in early stages of HD, i.e. low serum IGF1 and $^{31}$P-NMR spectroscopy (NMRS) in muscle. At baseline, $^{31}$P-NMRS displayed two patients with end-exercise muscle acidosis despite a low work output. On day 2, the introduction of triheptanoin was well tolerated in all patients, and in particular, there was no evidence of mitochondrial overload from triheptanoin-derived metabolites. After 4 days of triheptanoin-enriched diet, muscle pH regulation was normalized in the two patients with pre-treatment metabolic abnormalities. A significant increase of serum IGF1 was also observed in all patients (205 ng/ml ±60 versus 246 ng/ml ±68, p=0.010). This study provides a rationale for extending our anaplerotic approach with triheptanoin in HD.

## Introduction

Huntington disease (HD) is an inherited severely disabling disorder without curative or preventive treatment. We showed that early weight loss in HD is associated with a systemic metabolic defect, and that branched chain amino acids (BCAA) levels can be used as a biomarker, indicative of disease onset and early progression.[1] Levels of IGF1, which is regulated by BCAA, were also significantly lower in the HD group.[1] The decrease in plasma BCAA observed in our HD group suggested the activation of a compensatory mechanism to provide energy substrates to the Krebs cycle, as described in cachexia-producing illnesses.[2] Likewise, dietary compounds such as triheptanoin have been used in human therapeutic trials for their ability to refill the pools of intermediates of the Krebs cycle, a key energetic process called anaplerosis.[3] Because of additional evidence for triheptanoin metabolites to cross the blood brain barrier,[4] anaplerotic therapies represent promising molecules for reversing the energy deficit observed in both the brain and the periphery in HD patients. Based on the hypothesis that there is a need for Krebs cycle energy substrates in HD, we conducted a pilot study using a dietary anaplerotic approach to assess safety and short-term metabolic correction.

## Methods

*Study design*

We included six HD patients with abnormal CAG repeats expansions (mean= 46 ±3) in the *HTT* gene with a mild to moderate disease, measured by the clinical scale UHDRS (Unified Huntington Disease Rating Scale) of 33 ±13 (Table 1). All participants were enrolled in a clinical protocol promoted by INSERM (COS 07-26, http://bir.inserm.fr), and approved by the local ethics committee. Written informed consent was obtained for all participants.

On the screening day – approximately one month prior to the therapeutic trial – motor dysfunction was evaluated with the UHDRS, and each HD participant underwent $^{31}$P-NMRS testing in muscle. The six HD patients were asked to fill an opened-ended questionnaire during three consecutive days so that the dietician could determine caloric intake and devise daily menus ensuring that the triheptanoin oil provide 40% of an isocaloric diet, as previously established.[3,4] At day 1, the six HD participants were hospitalized and height and weight were recorded to calculate the body mass index (BMI). Blood and urine samples were collected after an overnight fast for standard analyses as well as plasma acylcarnitines, amino acids and ketone bodies, serum IGF1 and urine organic acids as described.[4] At day 2, HD patients ingested the first doses of triheptanoin (1g/Kg/day divided in four meals). Repeated blood samples were collected before and, sequentially, after the first meal (30, 60, 90, 120 and 180 minutes after triheptanoin ingestion) for assessment of acylcarnitines profile, amino acids and ketone bodies. Urine samples were collected before and after the first ingestion of triheptanoin (90 and 180

minutes) for analyses of organic acids. During the next 3 days, HD participants kept consuming an isocaloric diet enriched with triheptanoin (1g/Kg/day). At day 5, standard analyses were repeated fasting, as well as plasma acylcarnitines, amino acids and ketone bodies, serum IGF1 and urine organic acids. UHDRS scoring was performed and muscle $^{31}$P-NMRS was repeated. In view of the low risks to subjects participating in this protocol, continuous and close monitoring by the Principal Investigator was done on an ongoing basis during the days the patients were taking triheptanoin – almost all triheptanoin-derived metabolites being utilized 3 to 4 hours after its administration.[4]

*$^{31}$P-NMR spectroscopy in muscle*

NMR was carried out as described.[5] Data were analyzed semi-automatically to provide: perfusion time-course, PCr rephosphorylation time constant (τPCr) and end-exercise acidosis level (pH). Intensity of exercise (W/S) was determined by correcting work output (W, in Joules) developed during exercise to calf muscle area section (S, in cm$^2$).

*Statistics*

The data are expressed as the mean ± standard error of the mean. Paired t-tests (SPSS) were used to compare the values of plasma acylcarnitines, serum IGF1 and UHDRS before and after triheptanoin.

**Results**

Patients' BMI ranged from 19 to 25. There was no correlation between CAG repeats and metabolic measurement at baseline, except for an inverse correlation with plasma glutamine ($p<0.001$). All patients tolerated the treatment well and no adverse event was monitored during the trial and beyond. As a result of feeding triheptanoin, urinary excretion of derivatives of heptanoate oxidation were detected – pimelate, 6-hydroxyheptanoate, 3-hydroxypentanoate (BHP), 3-ketopentanoate (BKP), 3-hydroxypropionate, and methylcitrate – but there was no evidence of mitochondrial overload from triheptanoin-derived metabolites. Among plasma triheptanoin-derived metabolites, there was no substantial increase in either pentanoylcarnitine (C5) or heptanoylcarnitine (C7) (data not shown) but propionylcarnitine (C3) increased significantly in all patients (Table 1) indicating complete beta-oxidation of heptanoate to propionyl-CoA. Substantial increases of C5 ketone bodies were observed after meal and triheptanoin administration (data not shown), with BHP to BKP ratios between 2.1 to 2.8. Plasma BCAA initially decreased after triheptanoin administration and then returned to their baseline levels on day 5 (data not shown). There was no significant change in the UHDRS scores before and after triheptanoin (Table 1). There was no correlation either between CAG repeats and the levels of triheptanoin metabolites (data not shown).

During plantar flexion dynamic $^{31}$P-NMRS, all HD patients presented with a work output about two to four fold lower (Supplementary Table) than what is seen in healthy individuals – W/S= 34.96 J/cm$^2$ ±16.35, n=7. Measurements

of intramuscular pH showed that two patients (P5 and P6) presented with end-exercise muscle acidosis – pH at 6.72 and 6.84, Figure 1 – despite even lower exercise intensity compared to the four other patients – W/S at 11.44 $J/cm^2$ and 9.40 $J/cm^2$ respectively, Supplementary Table. Creatine rephosphorylation time constant was also increased in these two patients – tPCr respectively of 63.1 and 52.5s – but turned out to be normal when corrected for pH.[6] Of note, patient 5 had the lower BMI (i.e. 19) whereas patient 6 had the higher UHDRS score (i.e. 49).

Remarkably, after 4 days of triheptanoin-enriched diet, we observed a correction of end-exercise muscle acidosis – pH at 6.97 and 6.98, Figure 1 – in the two patients with pre-treatment metabolic abnormalities (P5 and P6). This was associated with different hyperemic responses after treatment with an earlier return to basal level of perfusion for patient 5 (Figure 2) and the occurrence of a distinct peak of functional hyperemia from the 3$^{rd}$ minute of recovery for patient 6 (Figure 2). In addition, we observed a significant increase of serum IGF1 in all HD patients (Table 1).

**Discussion**

Based on our observation of a hypercatabolic profile early in HD, our short-term study aimed at directly identifying an energy deficit followed by a therapeutic intervention to improve peripheral energy metabolism in HD patients. Our anaplerotic approach using dietary triheptanoin was well tolerated in all HD patients, both clinically and biochemically. Of note, the levels of plasma BCAA decreased temporarily after the initiation of triheptanoin before returning to their baseline levels, likely reflecting the reversible inhibition of branched chain $\alpha$-ketoacid dehydrogenase kinase by medium chain fatty acids.[7] Furthermore, we analyzed markers of peripheral energy metabolism that were shown to be altered in early stages of HD, i.e. low serum IGF1[1] or delayed phosphocreatine recovery (tPCr) after exercise measured by $^{31}$P-NMR spectroscopy in muscle.[8,9] Although $^{31}$P-NMR spectroscopy can lack sensitivity to detect mitochondrial dysfunction,[10] the most noticeable finding from our study was that two patients developed muscle acidosis at baseline while performing a low intensity exercise. Their end-exercise pH corresponded to W/S ratios three-fold higher than in normal subjects (data not shown). These two patients also had longer tPCrs but, when corrected for pH, tPCrs appeared to be unaltered.[6] This observation differs from previous observations although end-exercise pH was either not reported,[8] or unaltered in the HD group.[9] Of note, our anaplerotic approach with triheptanoin led to a normalization of end-exercise muscle acidosis in the two patients with pre-treatment abnormalities. NMR measurements of muscle perfusion also suggested an effect of triheptanoin on hyperemic responses to exercise. Moreover, the improvement of peripheral energy metabolism in our

study is supported by the significant increase of serum IGF1 in all HD patients. IGF1 impacts mutant huntingtin by promoting its phosphorylation. Phosphorylation inhibits mutant huntingtin toxicity by restoring its transport function.[11,12] Therefore, the increase of IGF1 following treatment with triheptanoin may exert direct beneficial effects by restoring huntingtin function and/or blocking huntingtin toxicity in peripheral organs and possibly in the brain. It is not clear how triheptanoin mediates IGF1 levels, especially since triheptanoin did not increase the levels of BCAA – known to regulate IGF1. Ketone bodies cross the blood brain barrier and ketogenic diet was shown to modulate IGF1 metabolism in the rat brain.[13] Likewise, it would be interesting to assess in vitro whether the ketone bodies derived from triheptanoin – i.e. BHP and BKP – can modulate IGF1 levels and huntingtin phosphorylation in primary cultures of striatal neurons.

In addition to the prominent role of brain energy deficit in the pathophysiology of HD,[14,15] there are several lines of evidence for a more systemic energy deficit. Our findings of hypercatabolism in HD[1] were indeed recently confirmed in both clinical [16,17] and preclinical studies.[18,19] Our data emphasize that $^{31}$P-NMRS may provide relevant biomarkers of peripheral energy metabolism in HD. This study also provides a rationale for extending our anaplerotic approach with triheptanoin in HD. A double-blind placebo-controlled trial with clinical outcome measurements is planned in order to confirm the potential benefit of anaplerotic therapies to improve, or even reverse, the energy deficit both in the periphery and the brain of HD patients and presymptomatic individuals.

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**Table 1:** Clinical characteristics of the six HD patients, as well as UHDRS, plasma propionylcarnitine (C3-carnitine) and serum IGF1 levels, before and after triheptanoin. Paired t-tests were used to compare the values before and after triheptanoin and significant changes are indicated with *p* values. * Pathological repeats size.

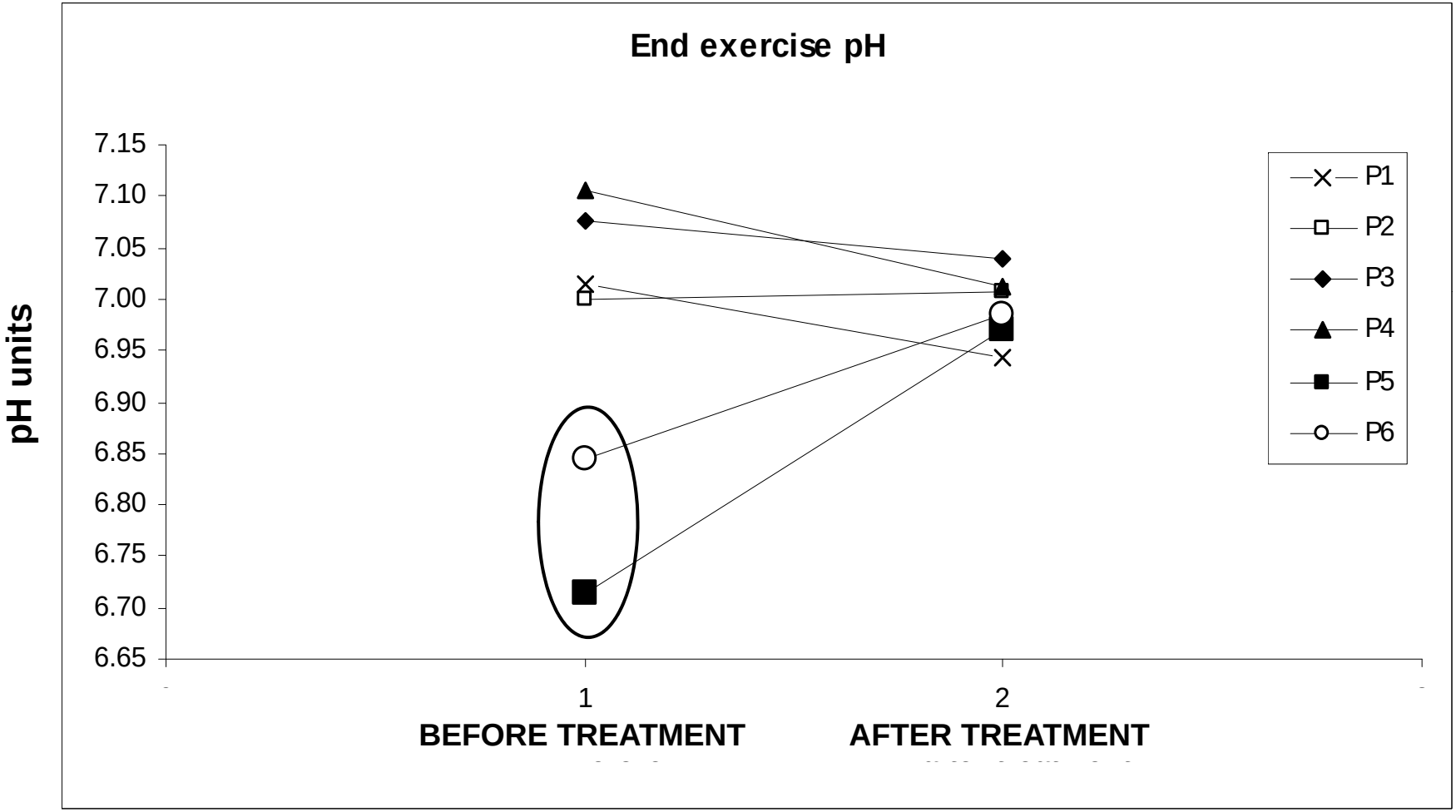
| | | | | | **UHDRS** | | **Plasma C3-carnitine (μmol/l)** | | | **Serum IGF1 (ng/ml)** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sex | CAG* | Age at onset (years) | Age at examination (years) | Pre-treatment | Post-treatment | Pre-treatment | Post-treatment | *p* | Pre-treatment | Post-treatment | *p* |
| **P1** | F | 46 | 49 | 51 | 39 | 43 | 0.17 | 0.52 | | 115 | 136 | |
| **P2** | M | 43 | 45 | 60 | 30 | 31 | 0.13 | 0.29 | | 196 | 268 | |
| **P3** | F | 51 | 30 | 34 | 43 | 38 | 1.16 | 2.85 | | 282 | 343 | |
| **P4** | F | 45 | 45 | 50 | 20 | 15 | 0.94 | 1.85 | | 238 | 254 | |
| **P5** | F | 43 | 40 | 48 | 15 | 9 | 0.52 | 1.78 | | 236 | 246 | |
| **P6** | F | 50 | 33 | 41 | 49 | 45 | 0.88 | 2.05 | | 165 | 218 | |
| **Total** | | 46 ±3 | 40 ±7 | 47 ±9 | 33 ±13 | 30 ±15 | 0.63 ± 0.43 | 1.56 ± 0.97 | ***0.011*** | 205 ± 60 | 246 ± 68 | ***0.010*** |

**Figure legends**

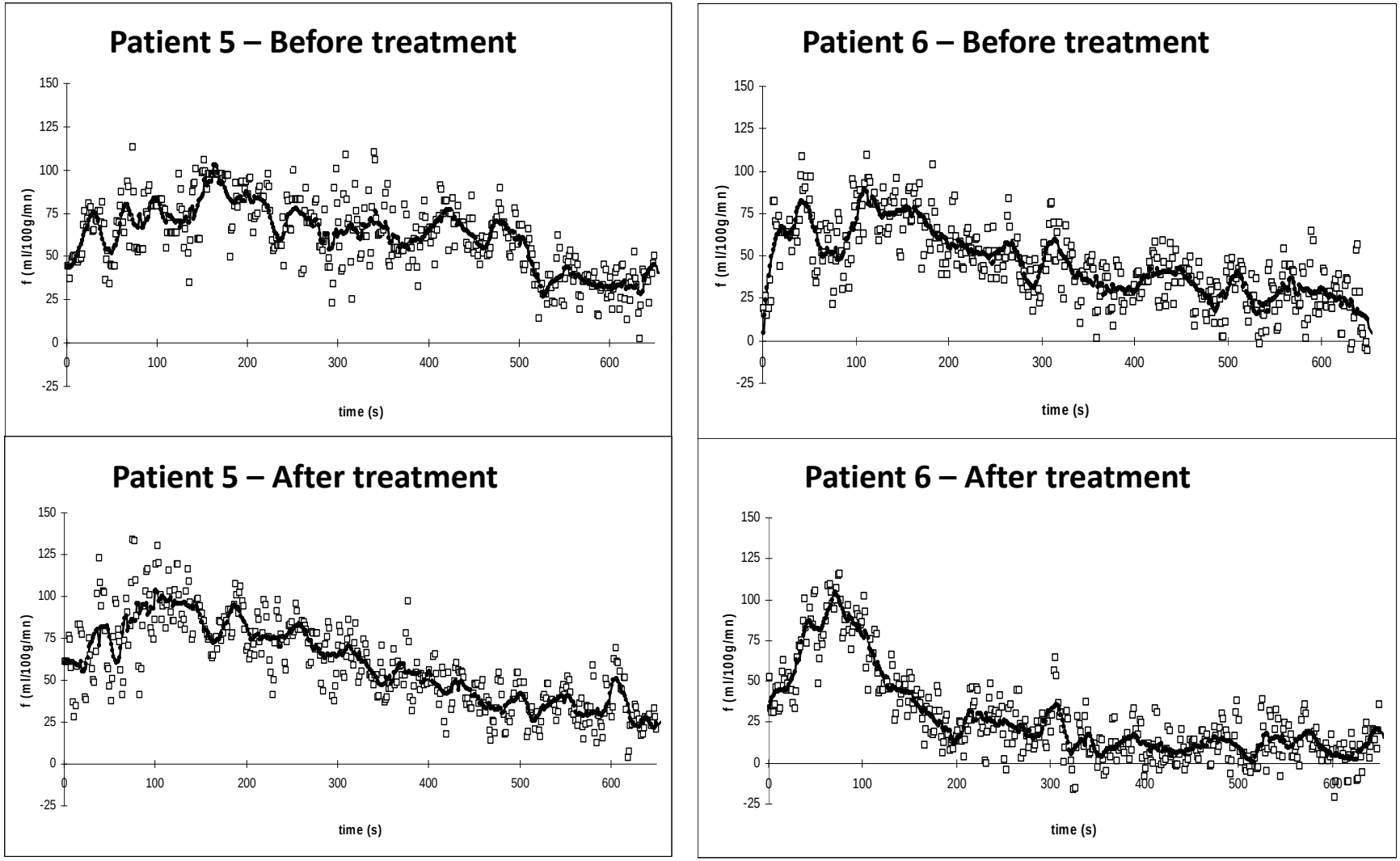
**Figure 1:** End-exercise pH before (1) and after (2) triheptanoin in 6 patients with Huntington disease (P1-P6). We observed normalization of the pH in the two patients (P5 and P6) with pre-treatment metabolic abnormalities.

**Figure 2:** Perfusion kinetics during exercise recovery before and after treatment in patients 5 and 6. The global hyperemic response was improved after treatment: more regular and rapid return to basal level for patient 5 (left panel) and distinction of a peak of hyperemia before reaching basal level of perfusion for patient 6 (right panel).

**Figure 1**


End exercise pH
pH units
7.15
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70
6.65
1
2
BEFORE TREATMENT
AFTER TREATMENT
P1
P2
P3
P4
P5
P6

# Figure 2


Patient 5 – Before treatment
f (ml/100g/mn)
time (s)
Patient 6 – Before treatment
f (ml/100g/mn)
time (s)
Patient 5 – After treatment
f (ml/100g/mn)
time (s)
Patient 6 – After treatment
f (ml/100g/mn)
time (s)